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(6) Extremely Low Frequency Electric Field Induced Changes in Rate of Growth and Brain and Liver Enzymes of Rats.

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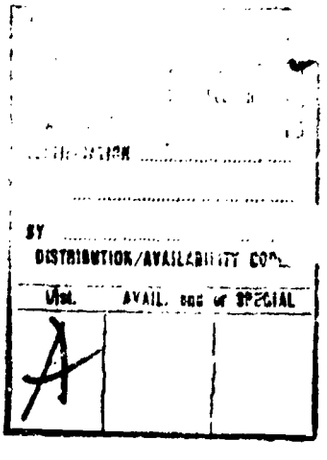
Abstract. Young adult male rats, maintained for 30 to 40 days in 45 Hz vertical electric fields varying from 100 V/m to 0.1 V/m, gained weight at rates 20 to 30% slower than control rats not exposed to 45 Hz radiations and had less abdominal fat deposits. Experiments were conducted in especially equipped railroad cars, which provided shielding from ambient electromagnetic fields. An effect on subcortical neurons was shown by a consistent decrease in activity of the neuronal enzyme choline acetyltransferase in the brainstem of rats maintained in 45 Hz fields, whereas, cerebral levels of this enzyme were not changed. Rats had elevated levels of liver tryptophan pyrrolase, but exhibited normal activity and eating and drinking behavior during maintenance in these fields.



Extremely low frequency (ELF; below 100 Hz) electric fields have been reported to have effects on a wide range of organisms, including amoebae (1), flatworms (2), slime molds (3), ringbilled gull chicks (4), guinea pigs (5), mice (6,7), rats (8, 9), monkeys (10) and humans (11,12). Other workers reported the lack of effects in humans (13) and rats (14) exposed to ELF for long periods. The extremely low energy content of ELF fields at the low intensities used in most of these reports, causes uncertainty concerning sensing mechanisms involved in the interaction of various organisms with ELF and the most appropriate ways to measure biological effects of ELF.

To seek possible mechanisms of interaction with ELF fields, we have studied various responses in male, Sprague Dawley rats maintained continuously in low intensity vertical electric fields with a frequency of 45 Hz, similar to fields proposed for use in long range systems of communications (15). Experiments were conducted within especially equipped, air conditioned railroad cars, which provided shielding from ambient ELF.

It was found that rats, maintained for 30 to 40 days in 45 Hz fields with intensities varying from 100 V/m down to 0.1 V/m (volts/meter, root mean square), had decreased rate of growth and reduced deposits of abdominal fat, compared to control rats. The neuronal enzyme, choline acetyltransferase, was consistently decreased in activity in the brainstem but not in the cerebral hemispheres of rats maintained in 45 Hz fields. This suggested that certain neurons in the brainstem were especially sensitive to the imposed 45 Hz fields. Adrenal weights were unchanged but the liver enzyme, tryptophan pyrrolase, was elevated in many of the groups of rats maintained in 45 Hz fields, which could indicate that the fields were able to produce a low level stress response in the rats.

After preliminary experiments using 100 V/m fields, several larger trials were run during which groups of rats were maintained in fields varying from 100 to 0.1 V/m. In each trial, three groups of rats were each exposed to a different 45 Hz field and a fourth group of control rats were protected from the imposed 45 Hz fields. Rats

were housed in polycarbonate cages within exposure chambers, received Purina Lab Chow and water ad libitum, and were weighed three times a week. The number of rats per cage in each of the trials was: Trial 1, 3 rats; Trial II, 1 rat; 2 rats per cage were used in the other experiments. Cages were open at top for air circulation, and of sufficient height to prevent rats from reaching the upper electrode plate. The polycarbonate cages insulated rats from the electrode plates. Electric fields were produced between 16 gauge aluminum plates below the cages and parallel plates above the cages made of copper screening. with a 13 inch space between plates. Ambient light levels, at approximately 25 foot-candles at cage floor level, were supplied by incandescent lamps. Temperatures within the cages were uniformly maintained at 72±2°F. The electric field was generated by an oscillator with high stability, fed to a large power amplifier, which excited transformers connected to the plates. A resistor, placed in series with each transformer provided the level of field required. Uniformity of fields between the plates was determined with a small ELF electric field probe constructed according to a design of the IIT Research Institute (16). Control rats were maintained similarly to experimental rats but were not exposed to 45 Hz fields because the plates above and below controls were shorted with a thick braided jumper. In addition, the groups of control and exposed rats were placed in each of the four areas possibly used for 45 Hz exposure in the various Trials, to control for possible differences in effects in different areas. A braided guard band, placed around the edge of each exposure area and electrically grounded, helped to maintain a uniform 45 Hz field in the exposure chambers. At the end of the exposure period, rats were sacrificed by brief exposure to ether or halothane and then decapitation between the hours of 8:00 to 10:00 A.M. to minimize effects of diurnal variation. Whole brains were rapidly removed and frozen on dry ice. Frozen brains were weighed and dissected into three parts while thawing over ice, by removing the cerebral hemispheres and cerebellum; the remaining part of the brain was referred to as the brainstem. Each brain part was weighed, homogenized while ice cold and analyzed individually for choline acetyltransferase

activity using the radioassay method of Schrier and Shuster (17). Liver and adrenals were also removed at time of sacrifice and weighed. Livers were quickly frozen on dry ice until analyzed individually for activity of tryptophan pyrrolase by the method of Feigelson and Greengard (18). Blood from severed neck vessels was collected in heparinized tubes and the plasma used for determination of corticosterone by the method of Mattingly as modified by Cope (19).

In three preliminary experiments, it was found that rats maintained in 45 Hz 100 V/m fields had significantly depressed weight gain compared to unexposed controls. In Experiment No. 1, eleven older rats with an initial weight of 440g, gained an average of only 40g during 32 days in a 45 Hz, 100 V/m field, whereas eleven control animals gained 140g during the same period. A similar effect was seen in Experiment No. 2, using younger rats (200g body weight initially): eleven rats gained 120g during 32 days in the field as compared to a gain of 200g by control rats. Experiment No. 3 had a cross-over protocol in which one group (EC) of 24 rats were kept in a 45 Hz, 100 V/m field for 20 days during which they only grew from 160 to 180g body weight, compared to the control (CE) group of 24 rats which grew from 170 to 280 grams during this period. At 20 days, the position of the rats was unchanged but the electrical leads were switched over. The previously unexposed rats (CE) were then exposed continuously to the 45 Hz field for 32 days during which time they only gained an average of 10g (from 280 to 290g). The previously exposed group (EC) had no 45 Hz field imposed on it during this second 32 day period and rapidly started to gain weight at an almost linear rate from 180g to 390g. Since removal of the imposed 45 Hz field allowed the rats to gain weight at a normal rate, it was apparent that the depression of weight gain was associated with the presence of the 45 Hz field and was rapidly reversible.

The mean weights of groups of rats in four larger Trials and the mean gain in body weight during exposure to 45 Hz fields varying in strength from 100 V/m to 0.5 V/m are presented in Table 1. The percent decrease in average daily weight gain of all exposed rats in each Trial as compared to that for the control group in the same Trial was:

Trial 1, 30% decrease; Trial II, 30% decrease; Trial III, 20% decrease; Trial IV, 23% decrease. In a subsequent Trial a similar effect was observed when the field intensity was lowered to 0.1 V/m. Groups of 32 rats each, with a starting mean weight of 135g were studied. The group maintained for 30 days in a 45 Hz, 0.1 V/m field, gained only 100g to a final mean weight of 235g. In contrast 32 unexposed control rats grew to a mean weight of 275g, a gain of 140g during the same period.

Since there was a 1,000 fold difference in field strengths used (100 V/m to 0.1 V/m), this data shows a lack of a dose-response relation between decrease in weight gain and field strength. Upon autopsy, control unexposed rats were found to have large deposits of fat in the abdominal area which are generally found in rats at those ages. In contrast, it was consistently observed that abdominal fat deposits were barely visible or absent in rats which had been maintained in 45 Hz fields. This may have accounted for much of the difference in body weights between the two groups.

Other than the decrease in weight gain, rats exposed to 45 Hz fields appeared and behaved as healthy as the control rats. Ad libitum food and water intake by exposed and control rats were similar and did not account for the large weight gain differences. To study this, ten rats were exposed to a 6 V/m, 45 Hz field, for 30 days in plastic metabolism cages; after five days the mean weight of exposed animals was significantly lower ($p < 0.005$) than similarly housed unexposed controls. The exposed rats consumed a little more food and water per gram of weight gained per day than did the control rats and also excreted a greater volume of urine. The results were essentially the same in a repeat experiment in which mean food and water intake were similar for 20 rats maintained 30 days in a 2 V/m, 45 Hz field, as compared to 20 control rats kept in simila cages.

The neuronal enzyme, choline acetyltransferase (ChAc), which synthesizes acetycholine, was determined in separate parts of brains of rats maintained 30 to 40 days in 45 Hz fields ranging from 100 V/m to 10 V/m (Experiments No. 1 and 2 and Trial II). Mean weights of total brains from exposed rats were not significantly different from that of control rats in each experiment, although the exposed rats had a significantly decreased

rate of body weight gain. Levels of ChAc activity were significantly reduced in the brainstem portion of brains from each of the groups of exposed rats in the three experiments (Table 2). In contrast, levels of this enzyme in the cerebral hemispheres of rats exposed to 45 Hz fields of 50 V/m to 10 V/m, were not significantly different from levels in the cerebrum of control rats (Table 2). Levels of ChAc in cerebellum were measured with less certainty, being about 1/10 the value in the brainstem, with no apparent differences between exposed and control rats. The brainstem contains the hypothalamus and other brain centers concerned with the regulation of pituitary trophic hormones, including corticotrophin and growth hormone (20), and with the regulation of fat mobilization (21).

Lott and McCain reported that the posterior hypothalamic area was an "electrosensitive area" in rats exposed to a pulsed electric field in shielded, Faraday cages; whereas the cerebral cortex of rats exposed to the same pulsed field showed weaker, erratic responses (9). Subcortical areas of macaque monkey brains also appeared to have sensitivity to ELF electrical fields which did not affect EEG activity in cortical areas (10). Since brain waves are predominantly in the ELF range, with a peak power output around 10 Hz (22) certain regions in the brain could be affected by external fields with similar frequencies. Bioelectric activities occur in many tissues, so it could also be postulated that tissues other than nervous tissues are capable of responding to ELF fields which are imposed on the whole animal.

As indicators of a possible stress response, determinations were made of the liver enzyme tryptophan pyrrolase, plasma corticosterone and adrenal weights in each of the rats maintained in 45 Hz fields and control rats which are listed in Table 1. In Trials II, III and IV, the control groups each had a level of liver tryptophan pyrrolase which we considered to be an acceptable control rat liver value based on other experiments with this enzyme. Eight of the nine groups of rats, maintained in 45 Hz fields in these Trials, had significantly elevated liver tryptophan pyrrolase levels (Table 3). In Trial I, the control group of rats as well as the three exposed groups of rats had levels of tryptophan pyrrolase above that expected for control rats. Elevations in this liver enzyme may occur in response to various types of stressful conditions (23).

Plasma corticosterone was measured on many of the rats exposed to 45 Hz fields from 100 V/m to 2 V/m. In Trial I control rats had a mean plasma corticosterone of 21 ± 12 µg/100 ml, whereas corticosterone values were significantly higher ($p < 0.05$) in groups of rats maintained in 45 Hz fields of 100 V/m (27 ± 15 µg/100 ml) and 50 V/m (33 ± 14 µg/100 ml). Plasma corticosterone levels in rats maintained in 45 Hz fields at 25 V/m or lower were not found to be significantly different from those of control groups of rats. Adrenal pairs were weighed for all of the rats listed in Table I. There were considerable variations in the values and they did not show a difference between rats maintained in 45 Hz fields and unexposed control rats. The mean adrenal pair weights for the three groups of exposed rats in each Trial and the control group in that trial were as follows: Trial I, exposed-54 mg, control-56 mg; Trial II, exposed-47 mg, control-49 mg; Trial III, exposed-50 mg, control-51 mg; Trial IV, exposed-70 mg, control-65 mg. The ratio of adrenal to body weight in the exposed rats was not statistically significant from that in controls. Stress responses that may have occurred in exposed rats were not sufficient to enlarge their adrenals as may occur in rats during chronic stress.

Our findings are consistent with those of Marino, Becker and coworkers (8), who studied rats maintained for one month in 60 Hz, 15 kV/m, vertical electric fields. In four experiments, a mean decrease in body weight gain was observed in the total of 56 rats exposed to 60 Hz fields as compared to 70 unexposed control rats. The difference in weight gain was significant ($p < 0.05$) in two of the experiments. Serum content of adrenal corticoids and blood proteins were consistently altered in the exposed rats. These workers also reported that mice maintained for three successive generations in 60 Hz, 10 kV/m fields, had significantly decreased body weight gain at 35 days postpartum compared to control mice. Decreased weight gain extended into the third generation of mice raised in vertical 60 Hz fields and into the second generation raised in horizontal 60 Hz fields (7).

Mathewson et al studied rats maintained for 30 days in vertical 45 Hz fields with intensities of 100 V/m to 10 V/m. Although the experimental conditions used by these

investigators were similar in many aspects to those used in our experiments, they reported finding no alteration in growth rate of rats maintained in 45 Hz fields, as compared to unexposed controls (14). There also were no changes produced by maintenance in 45 Hz fields in complete blood counts, selected biochemical assays of plasma, or histological examination of various organs of rats. Our knowledge of variables that can influence these types of experiments, may not be sufficiently advanced at this time to explain differences in data obtained in different laboratories. One possible source of such variables are interactions that may have occurred with ELF fields in the environment from natural and man-made sources. This is particularly a consideration at the low field intensities used in these experiments. Shielding from ambient ELF, by the steel structure of railroad cars used in our experiments, possibly could account for differences between our data and that obtained by Mathewson et al (14), who did not use such shielding in their experiments. Shielding from ambient electromagnetic fields has been reported to alter normal physiological functions. Lang and Altman and coworkers at Saarbrucken showed that maintenance for two weeks in Faraday cages produced abnormal electrolyte and protein patterns in the blood of mice and guinea pigs; imposition of a 10 Hz vertical electric field on animals in Faraday cages corrected the physiological abnormalities (5,6). Wever and colleagues reported changes in physiological circadian rhythms in humans when living in an underground bunker constructed to shield out ambient electromagnetic fields. The imposition of a 10 Hz field on humans in the shielded bunker, produced normalization of the physiological functions studied (11). Although the interactions of living organisms with ambient ELF fields are poorly understood, it is feasible that rats, which are shielded from such external fields, could detect and respond to experimentally imposed, low intensity ELF fields as was observed in our experiments. Unshielded rats might be less responsive to such experimental fields. A field of 0.1 V/m may be relatively strong when compared to the smaller electrical output of brain (22). This consideration taken together with our data suggests that the lower threshold of effects from 45 Hz fields, which was sought in our experiments, might be several

fold lower than the lowest field intensity (0.1 V/m) studied in our shielded investigational area.

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Table 1. Changes in body weight of rats maintained in 45 Hz vertical electric fields for 30 to 40 days. In each trial three groups of rats were maintained in 45 Hz fields in polycarbonate cages, so that the rats had no contact with electrified plates above and below the cages; and a fourth group of control rats was similarly housed but without exposure to an imposed 45 Hz field. The three groups of rats in each trial were exposed to different field intensities. Average changes in rate of body weight gain in the groups of rats maintained in different 45 Hz fields within each of the trials were similar, so the overall average gain in weight for the three groups of rats is presented as compared to the control group for that trial.

Trial no.	Fields (V/m) imposed per group	No. rats Total	No. rats Per group	Exposure time (days)	Body wgt. (Mean + S.D.) Final (g)	Body wgt. (Mean + S.D.) Wt.gain (g) in field
I	100,50,25	143	48	36	342 ± 12*	142 ± 15*
	Control	47	47	36	414 ± 17	209 ± 20
II	50,25,10	47	16	40	335 ± 12*	150 ± 19*
	Control	16	16	40	400 ± 10	215 ± 11
III	10,6,2	94	32	30	269 ± 9*	131 ± 12*
	Control	32	32	30	304 ± 8	166 ± 12
IV	2.0,1.0,0.5	94	32	30	263 ± 10*	131 ± 11*
	Control	32	32	30	302 ± 8	170 ± 11

* $p < 0.001$ vs control group

Table 2. Choline acetyltransferase activity in brainstem and cerebrum of rats maintained in 45 Hz fields 30 to 40 days. Rats in Experiments 1 and 2 were separate groups treated as were rats in Trial 1 in Table 1. Rats in Trial II were selected at random from rats in Trial II (cf. Table 1). Units of enzyme activity = n mol acetylcholine formed per hr. per mg liver wet weight ± S.D. Whole homogenates of brain parts were used in the radioassay (17).

	No.rats in	Choline acetyltransferase activity				
Experiment	each field	Control	100V/m	50V/m	25V/m	10V/m
Exps. 1 & 2						
Brainstem	22	1.75	1.45*			
		±.19	±.19			
Trail No.II						
Brainstem	16	1.60		1.37*	1.32*	1.35*
		±.22		±.19	±.12	±.15
Cerebrum	16	1.62		1.62	1.52	1.52
		±.17		±.27	±.27	±.20

*Statistically different from Control ($P < 0.01$)

Table 3. Liver tryptophan pyrrolase activity in rats maintained in 45 Hz fields at various voltages. Duration of exposure to 45 Hz fields and numbers of rats in each group are given in Table 1. Units of enzyme activity = μ moles kynurenine formed per gm liver (ww) per hr ± S.D.

Trial	Field strengths			
II	Control	50 V/m	25 V/m	10 V/m
	1.08	1.32*	1.29	1.21*
	±.28	±.39	±.41	±.27
III	Control	10 V/m	6 V/m	2 V/m
	1.09	1.81*	2.33**	2.61**
	±.79	±1.01	±1.04	±.66
IV	Control	2 V/m	1.0 V/m	0.5 V/m
	1.01	1.91**	1.61*	1.61*
	±.46	±.58	±.40	±.40

* Significantly different from control, $p < 0.01$

** Significantly different from control, $p < 0.001$

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Young adult male rats, maintained for 30 to 40 days in 45 Hz vertical electric fields varying from 100 V/m to 0.1 V/m, gained weight at rates 20 to 30% slower than control rats not exposed to 45 Hz radiations and had less abdominal fat deposits. An effect on subcortical neurons was shown by a consistent decrease in activity of the neuronal enzyme choline acetyltransferase in the brainstem of rats maintained in 45 Hz fields, whereas, cerebral levels of this enzyme were not changed.

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